PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Boyse et al.	:	Group Art Unit: 1804
Serial No.: 08/442,277	:	Examiner: Stanton, B.
Filed: May 16, 1995	:	
For: ISOLATION AND PRESERVATION OF FETAL AND NEONATAL HEMATOPOIETIC STEM AND PROGENITOR CELLS OF THE BLOOD	:	
Attorney Docket No.: 6287-026	:	Pennie & Edmonds 1155 Avenue of the Americas New York, New York 10036-2711

SUPPLEMENTAL RESPONSE UNDER 37 C.F.R. § 1.115

Assistant Commissioner for Patents
Washington, D.C. 20231

S I R :

To supplement and pursuant to the Amendment filed by Applicants on October 30, 1996 in connection with the above-identified application, Applicants submit herewith a Terminal Disclaimer Under 37 C.F.R. § 1.321(b), accompanied by the appropriate fee, which was executed on November 6, 1996 by Christopher Moller, President of Biocyte Corporation, assignee of the above-identified application.

Applicants respectfully request that the executed Terminal Disclaimer be entered and made of record in the instant application, and that the rejection of claims 60-62, 67-102, and 104-111 for obviousness-type double patenting be withdrawn.

Respectfully submitted,

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-- BY HAND --

Lorenz et al., 1951, J. Natl. Cancer Inst. 12:197-201, documents the need as early as 1951[1] of an efficacious source capable of bestowing hematopoietic reconstitution (in this case, upon a subject suffering from irradiation injury).

The following publications also document the recognized need in the art for therapeutic methods that are capable of effecting hematopoietic reconstitution, particularly in patients exposed to radiation or chemotherapy as well as the problems associated with the use of bone marrow, including GVHD in allogeneic procedures, host vs. graft disease in allogeneic procedures; necessity for an invasive collection procedure in ill patients in autologous procedures, and the threat of contamination with malignant cells of the patient in autologous procedures, etc.: Thomas et al., 1957, N. Engl. J. Med. 257:491-496; McGovern et al., 1959, N. Engl. J. Med. 260:675-683; McFarland et al., 1959, Blood 14:503-521; Clifford et al., 1961, The Lancet: 687-690; Pegg et al., 1962, Br. J. Cancer 16:417-435[2]; Kurnick, 1962, Transfusion 2:178-187; King, 1961, J.A.M.A. 177:610-613.

Kurnick et al., 1958, Ann. Int. Med. 49(5):973-986, documents the need for an effective method of hematopoietic reconstitution for cancer patients:

> Because of the severe hematopoietic depression which results from rapid, intensive irradiation of the thoracolumbar region, potentially curable malignancies (particularly testicular seminomas and embryonal carcinomas, even with metastases) are often inadequately irradiated. The likelihood of salvaging such potentially curable cases would be considerably enhanced if irradiation could be pursued with less concern for the ensuing hematopoietic depression.

(p. 984).

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1 Furthermore, the idea of beneficially affecting survival of irradiated subjects by injecting bone marrow is attributed to a 1948 document by Rekers (reference 4).

2 Pegg et al. also disclose a fetal liver transplant that failed to afford hematopoietic reconstitution (p. 430, line 2).

This article also documents the problems with GVHD and host vs. graft disease in the use of allogeneic bone marrow transplantation, and the problems associated with the use of autologous ("autogenous") bone marrow due to its limited applicability and threat of contamination with malignant cells:

> Even with successful transplants, the problems remain of late irradiation deaths from the high dosage required, or production of antibodies against the host by the homologous marrow. [citation] Van Bekkum and Vos [citation] have confirmed late irradiation lethality. . . . Antibody production, either from the host against the graft or from the grafted tissue against the host, may be involved in the delayed mortality. [citation] It appeared to us that these problems could be avoided by the use of autogenous bone marrow. This would, in general, limit the application to individuals in whom bone marrow infiltration by malignant disease had not occurred.

(p. 974)

Newton et al., 1959, Brit. Med. J. 1:531-535, also documents the long-felt need for hematopoietic reconstituting cells to restore hematopoiesis after chemotherapy or irradiation of cancer patients, and the problem in using autologous bone marrow, *inter alia*, of contamination with malignant cells (see in particular, p. 531, first and third paragraphs, p. 535, col. 1, second and third paragraphs under Discussion, and col. 2, fourth and fifth full paragraphs).

Thomas and Storb, 1970, Blood 36(14):507-515, document the earlier, general lack of success in efforts to achieve hematopoietic reconstitution using bone marrow, until developments recent to 1970 (p. 507, first paragraph), and that the disclosed technique for human marrow aspiration suffers from the problems of retrieval of inadequate marrow cell numbers[3] and GVHD[4].

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[3] "Clearly, more marrow cells would be desirable." (p. 512, last line).

[4] See p. 513, fourth full paragraph, first sentence.

Tulunay et al., 1975, Proc. Natl. Acad. Sci. USA 72:4100-4104, is an investigation in a mouse model of the use of allogeneic fetal liver cells for hematopoietic reconstitution, and evidences the need felt in the art for methods of achieving hematopoietic reconstitution in humans with reduced GVHD (see, in particular, p. 4103, col. 2).

Valeri, 1976, in *Blood Banking and the Use of Frozen Blood Products*, Ch. 1, CRC Press, pp. 1-7, cited by the Examiner, suggests the separation of whole adult peripheral blood into the three components of red blood cell concentrates, platelet concentrates, and plasma protein derivatives for storage for future use of these components and discloses freezing of these individual components as one option for preserving them in order "to eliminate much of the blood banking waste and . . . provide specific blood components" (p. 4, col. 1).[5] The long-felt but still unsolved need for an abundant, readily available source of cryopreservable stem cells capable of carrying out hematopoietic reconstitution is explicitly acknowledged by the authors:

> Surely it would represent a great advance in clinical medicine <u>if it were possible</u> to obtain a sufficient number of hematopoietic-repopulating stem cells from donors in a practical manner under sterile conditions, culture these cells in vitro to increase the yield, type them for the HL-A antigens, and freeze-preserve them for future use [citations].

(p. 4, col. 1; emphasis added).

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[5] It is noted that the authors state that freeze-preserved blood is a misnomer, since "[d]uring frozen storage only the red blood cells are preserved" (p. 1, col. 2).

Thomas et al., 1977, Blood 49(4):511, further documents the problems, associated with bone marrow transplantation, of GVHD[6], and complications associated with marrow procurement.[7]

Hershko et al., 1979, The Lancet: 945-947, documents the long-felt need for sources of cells, effective in carrying out hematopoietic reconstitution of a recipient, that are abundant, easy to obtain, and without the threat of malignant contamination, thus avoiding the recognized problems associated with bone marrow transplantation. Hershko et al. investigate adult peripheral blood as an alternative to bone marrow in carrying out hematopoietic reconstitution. As stated by Hershko et al. (p. 946, col. 2):

> [s]uch a method would be of considerable advantage over the use of bone-marrow cells since (1) peripheral W.B.C. [white blood cells] are much easier to obtain than bone-marrow cells; (2) repeated W.B.C. collection by leucopheresis can provide these cells in practically unlimited numbers; and (3) in patients with malignant disease and bone-marrow metastases, peripheral W.B.C. may still provide uncontaminated pluripotential stem-cells for autologous marrow reconstitution.

Hershko et al. disclose that the use of peripheral blood leukocytes failed to improve marrow function, in contrast to the ability of bone marrow to provide marrow recovery.

Sarpel et al., 1979, Exp. Hematol. 7:113-120, further document the search for alternatives to bone marrow, since

> current limitations of the procedure preclude widespread clinical use. These include: 1) The need for donors that are identical at the major histocompatibility locus [citation]. 2) The need to procure cells by a surgical procedure on the donor which cannot readily be repeated. 3) Problems of

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6 See in particular p. 529, second and third paragraphs under "Discussion."

7 In particular, these problems are the need for a hospital stay, spinal or general anesthesia, postoperative pain and discomfort, occasional headaches, the need for repeat aspirations, and attention to yields of marrow obtainable (see p. 513, first and second full paragraphs).

> hematologic and immunologic support, prior to successful graft function [citation].
>
> . . .
>
> In addition, contamination of bone marrow aspirates with immunocompetent cells from peripheral blood may intensify graft versus host (GVH) reactions, while they may also provide for more prompt engraftment [citation].

(p. 113-114).

Thus, the authors investigate the use of peripheral blood mononuclear cells in a canine model, cautioning that "[t]he use of peripheral blood cells for allogeneic marrow transplantation may present an increased risk of severe GVH [graft versus host disease] based on the high proportion of T cells in the circulation" (p. 118), and that "[i]t remains to be seen whether the system will also provide for homologous grafting and possible manipulation of GVH reactions based on improved cell separation methods" (p. 118).

Gluckman et al., 1980, Brit. J. Haematol. 45:557-564, describes the attempted treatment of five patients with Fanconi anaemia (FA) by bone marrow transplantation (BMT). The results were poor. Only one patient survived for more than three years, while four patients died of acute severe GVHD soon after grafting. As stated by the authors, "[i]n this study, overall survival of bone marrow transplanted FA patients was unusually poor when compared to our own series of patients transplanted for severe aplastic anaemia . . . This difference could be due to chance . . . or to a special susceptibility of this group of patients to the transplantation manoeuvre. Others have reported some success in treating FA by BMT [citations], but it appears that they have encountered the same problems of toxicity of the conditioning regimen and early severe GVHD" (p. 561). They add that "GVHD was always present, it was unusually early and severe." (p. 562)

Touraine, 1980, Excerpta Medica Intl. 514:276-283, reports the simultaneous use of both fetal liver and thymus transplant in attempted hematopoietic reconstitutions of four patients with severe combined immunodeficiency diseases (SCID)[8], in view of the fact that for at least half of the infants suffering from SCID, there is no HLA identical bone marrow donor available (see p. 276).

Ochs et al., 1981, Pediatr. Res. (4 part 2):601 (Abstr. 952), compare repeated red blood cell transfusions, a single infusion of peripheral blood mononuclear cells (PBMC), treatment with fetal liver cells, or treatment with HLA-matched bone marrow in four patients with SCID associated with adenosine deaminase deficiency. Superior results were obtained with bone marrow: only the child treated with matched bone marrow displayed a normal antibody response (an immune system function). The authors state that "a full set of normal uncommitted lymphoid stem cells is required for complete immune reconstitution."

Touraine et al., 1983, Birth Defects: Original Article Series 19(3):139-142, report the results of fetal liver transplants (FLT) and/or fetal thymus transplants (FTT) in attempted hematopoietic reconstitution of patients with SCID. The authors note that FLT and/or FTT is used "when no compatible bone marrow donor is available" (p. 139, col. 1). The authors note that "the incidence of success obviously does not reach that of BMT [bone marrow transplantation] from an HLA-identical sib" (p. 141). GVHD is noted as a prevailing problem. Some of the practical questions disclosed as remaining ("optimal age of the fetus, optimal number of cells, . . . obtainment and preservation, . . . prevention of GVHD . . .") highlight some of the limitations in using FLT, *e.g.*, limited accessibility of fetal liver and GVHD.

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[8] One of the four patients is reported to have achieved full reconstitution.

Storb and Thomas, 1983, Immunol. Rev. 71:77-102 document the long-felt problems associated with allogeneic bone marrow transplantation, including the occurrence of GVHD[9], low number of available marrow cells[10], and finding suitable donors,[11] and attempts to solve them. Other sources of stem cells are not even mentioned as practical alternatives to bone marrow:

> Marrow transplantation, once considered a desperate form of therapy in endstage patients, can now be considered as the preferred treatment for a variety of diseases in patients under the age of 50 who have a suitable donor. . . . For patients with acute leukemia who have relapsed at least once, whether or not a subsequent remission has been achieved, marrow grafting is the only approach that offers long-term, disease-free survival with 10-30% apparent cures. Although still somewhat controversial, it appears that marrow grafting is also the treatment of choice for patients with acute, non-lymphoblastic leukemia in first remission and for patients with chronic myelogenous leukemia since approximately 50-60% of these patients are surviving in complete remission, apparently cured. Marrow grafting is the only effective treatment for many patients with immunologic-deficiency diseases (Pahwa et al. 1978).

(p. 93)

Gluckman et al., 1983, Brit. J. Haematol. 54:431-440; 1984, Sem. Hematol. 21:20-26; and 1985, The Cancer Bulletin 37:238-242, all describe a modified chemotherapeutic conditioning regimen with improved results in treating Fanconi anemia

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9 "Despite the initial restriction of the human donor-recipient pairs to HLA-identical siblings and the 'prophylactic' use of MTX, GVHD is a serious problem in humans." (p. 79, last paragraph); see also pages 79-85.

10 The authors disclose on page 86 that a low number of transplanted marrow cells was one of three factors associated with graft rejection in certain patients, graft rejection being the most frequent problem associated with high mortality in such patients before 1975. On page 87, the authors note that "[g]raft rejection is less likely when a larger number of marrow cells is transplanted" and disclose supplementation with adult peripheral blood cells since "[t]he donor's peripheral blood may serve as a source of additional, pluripotent, hematopoietic stem cells and/or lymphoid cells capable of overcoming rejection."

11 See p. 94.

(FA) patients with bone marrow transplantation. The articles note the occurrence of some GVHD, even in patients treated using the modified regimen. In the 1985 article, out of 13 patients treated using a modified conditioning regimen, acute GVHD was observed in nine patients, and chronic GVHD was observed in five patients (p. 240). In conclusion, in both the 1984 and 1985 articles, the authors state that "[b]one marrow transplant[ation] offers a good chance of cure providing that there is an HLA-identical healthy donor."[12]

Good et al., 1983, Cell. Immunol. 82:36-54, notes the still existing need for a source of stem cells with the efficacy of bone marrow in carrying out hematopoietic reconstitution, but that is abundant and readily available and/or exhibits reduced GVHD relative to bone marrow, in the context of treating children with SCID:

> It is essential only to provide these children the relatively few necessary stem cells. . . . one can prevent the otherwise regularly fatal graft-versus-host reaction in these children [with SCID] by transplantation of marrow from an HLA-matched sibling. . . . By contrast, if donor and recipient are not siblings matched at the MHR, transplantation of bone marrow to correct severe combined immunodeficiency regularly has led to a fatal outcome because of the severe graft-vs-host reaction that is induced [citations].

(p. 38).

The authors further note:

> The most pressing and often most difficult problems, however, are those associated with acute and chronic graft-versus-host reaction.

THE PROBLEM OF GRAFT-VERSUS-HOST DISEASE (GVHD)

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[12] Attention is invited to the multiple successes in treating Fanconi anemia by achieving hematopoietic reconstitution using the claimed methods of the instant application, and the advantages thus afforded in the use of cryopreserved stem cells from human neonatal/fetal blood, relative to bone marrow (*e.g.*, greater chance of finding an HLA-identical donor due to the greater availability and abundance of human neonatal blood, ease of collection, etc.). See *e.g.*, Gluckman et al., 1989, N. Engl. J. Med. 321:1174-1178; Gluckman et al., 1990, Bone Marrow Transplantation 5(Suppl. 2):42; Kohli-Kumar et al., 1993, Brit. J. Haematol. 85:419-422.

> GVHD even presents a difficult problem sometimes when a matched sibling donor is used for the marrow transplants.

(p. 42)

And elaborate further:

> However, one of the most pressing current questions with bone marrow transplantation remains, "How can one avoid graft-versus-host reaction?" These reactions produce disease that is featured by fever, maculopapular rash, hepatosplenomegaly, diarrhea, and pancytopenia. The reaction leads to disease that is often ultimately fatal [citations]. Both acute and chronic forms of graft-versus-host disease have been described and both of these diseases exhibit high morbidity and contribute to lethality of other complications of marrow transplantation, e.g., intercurrent infection with opportunistic pathogens.

(p. 44)

The authors then detail on pages 45-48 various methods used in attempts to reduce GVHD.

Good et al., 1983, Cell. Immunol. 82:36-54, also notes the reasons why fetal thymus and fetal liver have not been found to be satisfactory alternatives to bone marrow. With respect to fetal thymus, the authors state:

> A few cases of SCID have been described where thymic transplants have corrected the disease [citation]. However, in our experience, such corrections of SCID as achieved by Dr. Richard Hong and his colleagues are exceptions. Indeed we have recorded a number of instances in which efforts at thymus transplantation have been accompanied by sustained correction of thymus hormone levels to normal which did not correct the immunodeficiency in patients with SCID [citation]. Thus we do not feel that thymus transplantation for most patients with SCID will be a curative approach to their immunologic constitution.
>
> By contrast, when a matched sibling donor is available, SCID is regularly corrected by bone marrow transplantation.

(p. 39).

With respect to fetal liver, they state:

> We [citation], as well as our former associates, Touraine *et al.* [citation], and Cooper and co-workers [citation], have corrected the immunodeficiency of several cases of SCID using fetal liver or fetal liver plus fetal thymus transplants. However, this combination has been effective only about 25-30% of the time [citation].
>
> For several reasons, fetal liver has not been an entirely satisfactory source of stem cells. Using transplantation of spleen from neonatal animals or from thymectomized mice [citation], we further ascertained that if stem cells were obtained from any cell source that lacked post-thymic elements, fatally irradiated mice could be reconstructed hematopoietically and immunologically without producing GVHD. In all these experiments, both the committed immunoincompetent T cells and the fully differentiated immunocompetent T lymphocytes had to be absent from the stem cell source. If this was not the case, graft-vs-host reactions would be the consequence with any hematopoietic source.

(p. 45).

Herzig, 1983, in *Bone Marrow Transplantation*, Weiner et al. (eds.), The Committee on Technical Workshops, American Association of Blood Banks, Arlington, Virginia, pp. 123-146; hereinafter "Herzig"), evidences the long-felt needs for easily obtainable, free from contamination with malignancy or other adult disorders, sources of stem cells which can effect hematopoietic reconstitution with low incidence of GVHD. As described therein (pp. 123-124), autologous bone marrow transplantation (ABMT) was first introduced in 1958 in an attempt to provide a source of stem cells to restore hematopoiesis, with disappointing results over the next four years. Some success was achieved in 1977 by Thomas et al. with allogeneic BMT. However, as set forth therein, allogeneic BMT suffers from the "major problem" of "graft-vs-host disease (GVHD) which causes substantial morbidity and mortality"[13] even with histocompatible donors, as

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[13] Herzig, p. 124, lines 4-6.

well as the problems that "allogeneic BMT has limited applicability because the majority of patients do not have a histocompatible sibling donor."[14] Additionally, contamination with small numbers of tumor cells, undetected at the time of marrow storage, poses a potential problem with its therapeutic use[15]. Herzig also shows that as of its date of publication (1983), the use of adult peripheral blood mononuclear cells as an alternative source of hematopoietic stem cells for hematopoietic reconstitution was of dubious utility due to the failures which had been encountered. Even the initially encouraging results which had been obtained (followed by failure), were obtained using cells from a limited group -- patients with cancer that are believed to have a greatly expanded stem cell pool[16] by virtue of their treatment.

Herzig also documents that alternatives to bone marrow as a source of hematopoietic stem cells for hematopoietic reconstitution were desired in order to "avoid the discomfort of marrow donation"[17].

Herzig documents the needs in the art from the early reports (1958 and later) it mentions by reference, up to its date of publication, 1983. The many other publications in the art discussed herein further document the long length of time over which the art has searched for a useful, safe, effective, readily available and inexpensive means to achieve hematopoietic reconstitution.

Abrams et al., 1983, J. Cell. Biochem. Suppl. 7A:53 (Abstr. 128), document that there was by then a need, long felt by those skilled in the art, for

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14 Herzig, p. 123, last paragraph.

15 Herzig, p. 124, lines 11-15.

16 Herzig, p. 124, last paragraph spanning p. 125; see also the '681 Patent at col. 6, lines 1-14.

17 Herzig, p. 124, last paragraph.

alternatives to bone marrow which would overcome the problems of malignant contamination, prior irradiation or other effects of patient treatment, limited marrow availability, and the invasive procedure required for procurement:

> In the autologous setting the ability to collect marrow from the bony pelvis may be compromised by clinically evident tumor involvement of the pelvis bones, prior pelvic irradiation, or chronic treatment related marrow hypoplasia. The ability to effect autologous hematopoietic reconstitution through the use of infusions of peripheral blood mononuclear cells could in theory overcome these difficulties either by obviating the need for bone marrow harvesting or by augmenting limited marrow availability.

This need leads the authors to investigate the use of peripheral blood mononuclear cells (PBMC) in a canine model, leading to the demonstration that PBMC collected at the time of cyclophosphamide (a chemotherapeutic agent)-induced expansion of circulating progenitor cells can substantially augment reconstitutive potency of PBMC used alone (*i.e.*, not in combination with bone marrow), thus limiting the potential availability and benefits of PBMC as an alternative due to its temporally limited availability (and dependency on patient condition).[18]

Spitzer et al., 1984, Cancer 54:1216-1225, further documents the long-felt needs in connection with the use of bone marrow for hematopoietic reconstitution, not yet

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[18] As pointed out by Broxmeyer et al., 1989, Proc. Natl. Acad. Sci. USA 86:3828-3832:

> autologous or major histocompatibility complex-matched bone marrow transplantation is the usual therapeutic vehicle for hematopoietic reconstitution. Although adult blood has had some use as an alternative source (5), in practice the content of stem/progenitor cells is so low (1,2) that multiple leukopheresis is necessary and has been reported primarily in patients undergoing intense temporary rebound hematopoiesis resulting from recent chemotherapy (5-17).

(p. 3838, cols. 1-2).

To similar effect, see Broxmeyer et al., 1990, Int. J. Cell Cloning 8(Suppl. 1):76-91 at p. 77, second full paragraph.

solved by the art. With respect to allogeneic bone marrow transplantation, the authors note that "[t]he complications of allogeneic bone marrow transplantation are well described, the restrictions of available donors well appreciated, and the scarcity of identical-twin donors obvious" (p. 1216, col. 1). With respect to autologous bone marrow transplantation (ABMT), the authors note that this "poses a few problems different from that of allogeneic bone marrow transplantation, such as quality of bone marrow stem cells and bone marrow tumor contamination" (p. 1216, cols. 1-2), and that areas of importance in any discussion of autologous transplantation include "methods of purging bone marrow of contaminating tumor cells with either monoclonal antibodies, *in vitro* chemotherapy, or combinations of both" (p. 1216, col. 2). More details are then provided in the text of the article. The authors conclude that "[a]utologous bone marrow transplantation results should also improve over the next years with: (1) the improvement of purging techniques of bone marrow . . . " (p. 1223, col. 2).

O'Reilly et al., 1984, Sem. Hematol. 21(3):188-221, also documents the problems associated with bone marrow transplantation, particularly GVHD, and attempts to alleviate the same (see *e.g.*, pp. 188-190, 193, 202, 207-211), as well as problems with engraftment (see *e.g.*, p. 193). With respect to GVHD, the authors close by evidencing the still extant need for methods to reduce GVHD: "Continued improvements in methods designed to prevent or abrogate GVHD and to protect the transplant recipient from early life-threatening infections should insure a broad and increasingly successful application of marrow transplants for the curative treatment of these lethal or severely debilitating disorders" (p. 211).

Dicke et al., 1984, Sem. Hematol. 21:109-122, documents the problem of malignant contamination of bone marrow in autologous bone marrow transplantation (ABMT) of cancer patients. Attempted methods to eliminate these malignant cells in

marrow by *in vitro* manipulations prior to transplantation are described in detail, and the still existing need for better methods is noted (see pp. 110-114, 117, 118). With respect to acute leukemia, the authors state that "[s]tudies of autologous transplantation in acute leukemia demonstrate that leukemic cells contaminating the bone marrow may play an important role in the recurrence of leukemia after transplantation. . . . It may be worthwhile to investigate a number of other drugs that can be used for the elimination of leukemic cells." (p. 119). With respect to lymphoma, the authors state that "[t]he risk of bone marrow involvement and of circulating tumor cells is always present in lymphoma, especially in lymphoblastic lymphoma in which purging of marrow with tumor-reactive monoclonal antibodies or with chemical agents may be indicated" (p. 116). With respect to small cell bronchogenic carcinoma (SCBC), the authors note that high-dose chemotherapy in combination with ABMT will not be able to cure the majority of cases; it is stated to be uncertain whether this is due to "the fact that, in the majority of cases, marrow is contaminated with tumor cells," and that "[d]rug resistance of noneradicated tumor cells appears a high probability" (p. 117, last paragraph). With respect to neuroblastoma, the authors note that "[b]one marrow contamination with tumor cells exists in this disease. Therefore, purging methods [to remove malignant cells] for marrow cell suspensions to be transplanted need to be developed." (p. 118).

Juttner et al., 1985, Brit. J. Haemotol. 61:739-745, discloses that use of early remission autologous peripheral blood stem cells (PBSC) of patients with acute non-lymphoblastic leukemia yielded evidence of hematopoietic reconstitution, a finding only previously demonstrated convincingly in humans using PBSC from chronic granulocytic

leukemia patients (p. 743, first full paragraph). The potential problem of malignant contamination of the stem cells is noted.[19]

Prümmer et al., 1985, Exp. Hematol. 13:891-898, discloses that "the procurement of autologous bone marrow (BM) may be complicated, however, owing to the patient's general performance or as a consequence of prior treatment" (p. 891), and state that autologous peripheral blood leukocytes, as a source of stem cells alternative to or in addition to BM, may find use based on their studies in dogs.

Rowley et al., 1985, Exp. Hematol. 13:295-298, documents the recognition in the art that "[s]uccessful autologous marrow transplantation is currently limited by residual marrow tumor cells presumed present in certain neoplasms even when a clinical complete remission has been achieved and no detectable tumor persists at the time of marrow harvest" (p. 295, col. 1).

Kaizer et al., 1985, Blood 65:1504-1510, notes that: "The problem that prevents the more general use of cryopreserved autologous marrow is the presence of occult leukemic cells in remission marrow" (p. 1504, col. 1); the authors thus investigate the potential efficacy of purging leukemic cells from marrow by use of 4-hydroperoxycyclophosphamide (4 HC).

O'Reilly, 1985, in *Fetal Liver Transplantation*, Gale et al. (eds.), Alan R. Liss, NY, pp. 327-342, documents the problems associated with the use of fetal liver, *i.e.*, the numerous failures in achieving hematopoietic reconstitution, GVHD, and limited availability of sufficient fetal tissue, and shows that, in 1985, the use of fetal liver or fetal thymus transplants was not viewed as an efficacious alternative to the use of bone marrow

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[19] "The question of leukemic contamination of the PBSC remains to be answered." (p. 740).

transplants for hematopoietic reconstitution, and did not fulfill the needs long sought in the art:

> In the last 9 years we have accumulated a limited but significant experience with the use of HLA-mismatched fetal liver and thymus grafts and T-cell depleted HLA-haplotype disparate parental SBA^-E^- marrow grafts in the treatment of children with SCID. A comparison of our results with these 2 techniques (Table 1) and a review of the attributes of each approach has underscored several limitations to the use of fetal tissue transplants and led us to abandon this approach for all but exceptional cases.
>
> A major limitation to the use of fetal tissue grafts is their limited accessibility. In murine models the potential of fetal hematopoietic and lymphoid cells to induce severe GvHD has been correlated with the development of the thymus and its infiltration with lymphocytes, an event which, in man occurs between the 12th and 14th week of gestation. Indeed, lethal GvHD has been observed in recipients of fetal thymus of 16 weeks gestation. Thus, fetal tissues should be of less than 12 weeks gestation if serious GvHD is to be avoided. However, elective abortions at this stage rarely yield intact fetuses suitable for procuring fetal liver or thymus. While large banks have been developed to cryopreserve fetal liver and thymus for transplantation purposes, to our knowledge, no human has ever been demonstrated to have been engrafted with cryopreserved fetal liver or thymus, suggesting that these cells when cryopreserved may be considerably more fragile than their marrow counterparts. Because of limited accessibility, fetal tissue grafts may be delayed for months before a suitable graft is available. The prospect of such delays almost prohibits the pretransplant use of cytoreductive agents to overcome host resistance and thereby potentiate engraftment.

(p. 335) (emphasis added).

O'Reilly further states:

> Engraftment of fetal liver with or without thymus is less consistent and much slower when achieved, than that observed following SBA^-E^- parental marrow transplants. This disadvantage of fetal liver/thymus grafts likely reflects, in part, the greater degree of genetic disparity existing between fetal donor and host and possibly other variables such as the smaller number of mature T cells in the fetal liver/thymus graft, or damage to fetal cells due to hypoxia

and liver cell lysis between the time of death of the fetus and its subsequent abortion.

. . .

An analysis of the functional reconstitutions of cell-mediated immunity achieved by patients durably engrafted with fetal liver/thymus or with $SBA^{-}E^{-}$ T-cell depleted parental marrow again strongly favors the use of the latter transplant approach.

. . . most recipients of fetal liver and thymus grafts have achieved only a limited reconstitution of T cell functions.

(p. 336-337).

O'Reilly concludes with the many significant advantages in the use of bone marrow relative to fetal liver/thymus:

. . . comparing the 2 approaches, transplants of T-cell depleted parental marrow, which are at least haploidentical with the recipient, appear to offer significant advantages over fully allogeneic fetal liver and thymus grafts in their accessibility, consistency and speed of engraftment, and the quality of the T cell reconstitution usually achieved. The superior accessibility of T-cell depleted parental marrow grafts also permits the use of cytoreductive agents to prepare the patient with SCID for transplantation, thereby enhancing the possibility of engrafting donor B lymphocyte precursors and achieving reconstitution of both T and B cell function in the post-transplant period.

(p. 339-340).

McGlave et al., 1985, in *Recent Advances in Haemotology*, Hoffbrand (ed.), Churchill Livingstone, London, pp. 171-197, is another review that documents the by then (1985) long felt but still unsolved needs associated with bone marrow transplantation -- to eliminate the problems of malignant cell contamination of marrow, GVHD, and finding suitable marrow donors. As the authors summarize in their introduction (and then elaborate in detail in the text which follows):

> Attempts to prolong survival substantially in patients with malignancy who receive intensive cytoreduction followed by infusion of autologous marrow have not been rewarding; however, methods to 'purge' malignant cells from autologous marrow prior to reinfusion are being investigated.
>
> The most important obstacles to the development of BMT [bone marrow transplantation] have been infection and graft versus host disease (GVHD). Progress has been made in the prevention and treatment of infection in this uniquely immunocompromised population, and in the understanding of the pathophysiology and clinical course of GVHD. Innovative approaches to the prevention of GVHD including in vivo or ex vivo removal or inactivation of donor T-lymphocytes with biological or chemical agents are being investigated.
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> At present, allogeneic BMT is confined to patients with siblings matched at the major histocompatibility loci (MHC). Efforts are underway to use selectively mismatched, related donors after manipulation of donor marrow. Programmes are also being designed to find unrelated, MHC-matched donors from the general population.

(p. 171).

Coulombel et al., 1985, J. Clin. Invest. 75:961-969, shows that in 1985 a solution to the problem of malignant contamination in autologous bone marrow samples was still being sought:

> . . . it is already clear from studies of treated AML [acute myelogenous leukemia] patients that in many instances hemopoiesis in remission is regenerated from coexisting nonclonal stem cells (1). In fact, this has stimulated efforts to develop a variety of methods for removing residual leukemic cells from AML marrow samples destined for autologous reinfusion following treatment of subsequent relapses. In view of current technical and practical problems facing investigators in this area, selection in favor of normal elements using the type of culture procedure described here offers an alternative that may be worthy of future consideration.

(p. 968, col. 1).

Caistaigne et al., 1986, Brit. J. Haematol. 63(1):209-211, also report the use of adult autologous PBMC in a patient with acute leukemia to achieve hematopoietic reconstitution. The authors note that:

> The successful haematopoietic reconstitution which occurred was related to a sufficient number of circulating stem cells collected by CFL during the early phase of remission following chemotherapy. This contrasts with the previously reported failure of this technique in man when CFL was performed in a normal subject, i.e. an identical twin. In these cases the number of CFU-GM transfused was either very low (Abrams *et al*, 1980) and/or administered in fractions over many days (Hershko *et al*, 1979). Moreover, there is no clearcut relationship in man between the numbers of CFU-GM and pluripotent stem cells. In our patient the CFU-GM obtained through the three CFL were as numerous as those usually infused for autologous bone marrow transplantation. The sufficient number of circulating stem cells may be related to the fact that CFL were performed early during aplasia recovery.

(p. 210).

Thus, the limitations of this method are apparent (see also Korbling et al., 1986, Blood 67(2):529-532).

Gorin, 1986, Clin. Haematol. 15:19-48, similarly documents the long-felt need for efficacious sources of stem cells capable of carrying out hematopoietic reconstitution (see, *e.g.*, paragraph spanning pp. 19-20), and the fact that the use of adult peripheral blood stem cells (PBSC) has not been an overall satisfactory alternative. With respect to PBSC, it is disclosed that success has been consistently achieved only with autologous PBSC from patients having chronic granulocytic leukemia (CGL) receiving blood containing very high doses of progenitors, and a recent successful autograft has been reported using autologous PBSC from a patient in remission from acute non-lymphocytic leukaemia (Reiffers et al., 1985, 11th Ann. Mtg. European bone marrow transplantation group, abstract 13) (p. 32, first four paragraphs). The author concludes

his article by expressing dissatisfaction with PBSC as an alternative to bone marrow: "[p]roblems associated with autografting with peripheral blood stem cells and cryopreserved CGL marrows should be a warning to all those involved in the field."

Korbling et al., 1986, Blood 67:529-532, also document the search for an alternative to bone marrow for hematopoietic reconstitution, and therefore investigate the use of autologous adult peripheral blood mononuclear cells (PBMC). As explained therein, the use of adult PBMC suffers from the following: its utility appears to be limited to autologous PBMC collected from certain cancer patients whose blood is in the "rebound" phase subsequent to chemotherapy, and thus the use of such PBMC suffers from limited availability with respect to both patient type and time of collection, is dependent upon patient condition, and suffers from the threat of malignant contamination.[20] The problems that have been experienced using adult PBMC, of insufficient cell numbers and GVHD, are also noted by Korbling et al. (p. 531, col. 2). Thus, the use of adult PBMC does not fulfill the long-felt needs associated with the use of bone marrow.

Stiff et al., 1986, Exp. Hematol. 14(6):465 (Abstr. 311) and Juttner et al., 1986, Exp. Hematol. 14(6):465 (Abstr. 312) also report attempts at hematopoietic reconstitution using cryopreserved autologous adult peripheral blood cells. Both articles report collection by leukapheresis after myeloblative therapy. Stiff et al. remark that "[t]he phereses were done during induction chemotherapy . . . and were timed to take advantage of the up to 14 fold increase in blood stem cells which occurs just after the blood count nadir." Based on their results, Stiff et al. note that the use of blood stem cells after supralethal therapy "fails to adequately restore thrombocytopoiesis." Two out

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[20] *See, e.g.*, p. 531, first full paragraph, regarding tumor cell presence in stem cells collected from patients with chronic myelogenous leukemia, and other references discussed herein.

of the four patients treated by Juttner et al. experienced relapses. Juttner et al. note that "[t]he minimum number of PBSC [peripheral blood stem cells] required for sustained engraftment is unknown, but it seems that the ratio of pluripotent stem cells to committed progenitors is lower in VER PB [very early remission peripheral blood -- used by Juttner et al.] than in either allogeneic bm [bone marrow] or autologous bm collected later in stable remission."

Tilly et al., 1986, The Lancet:154-155, also report that "[o]ne problem with peripheral blood cells is the collection of sufficient stem cells" (p. 154) and describe collection during bone marrow recovery after induction therapy in patients with acute leukemia in order to increase levels. The authors acknowledge the ensuing inherent limitation in that "[t]he timing of the collection of PB-HSC [peripheral blood haemopoietic stem cells] is important" (p. 155), i.e., that it be done at the immature cell peak after induction therapy.

Cain et al., 1986, Transplantation 41:21-25, disclose the occurrence of myasthenia gravis and polymyositis in a dog transplanted with fetal liver cells in which sustained engraftment with recovery of hematopoiesis was achieved, that was not associated with other features of GVHD. The authors note that GVHD "occurs frequently following hematopoietic transplantation in man" (p. 21) and that:

> [f]etal liver cells have been proposed as an alternative source of hematopoietic cells for transplantation. These cells have only a limited potential to cause graft-versus-host disease.[21]

(p. 24).

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21 Note, however, that Touraine et al., 1983, Birth Defects: Original Article Series 19(3):139-142, discussed *supra*, discloses problematic GVHD associated with the use of fetal liver in humans (see also Good et al., 1983, Cell. Immunol. 82:36-54 discussed *supra*).

The authors thus document the long-felt need for alternatives to bone marrow transplantation in humans which display reduced GVHD.

Yeager et al., 1986, N. Engl. J. Med. 315(3):141-147, documents the recognized problems, associated with allogeneic bone marrow transplantation, of GVHD and opportunistic infections, accounting for most of the subsequent mortality, and the lack of a suitable donor for 60-75% of leukemia patients (p. 141, col. 1). The publication also documents the recognized problem, associated with autologous bone marrow transplantation, of the possibility of contamination of the marrow with residual cancer cells, as well as the attempts which have been made that may have alleviated, but not eradicated, this problem (p. 141, col. 2, and p. 146, col. 2).

Reiffers et al., 1986, Exp. Hematol. 14:312-315, note the problem that use of autologous marrow transplantation:

> could be limited by the presence of neoplastic cells in the infused marrow. A variety of techniques has been proposed for the removal of leukemic cells from the marrow, but the clinical value of several of these methods has not been assessed yet. . . . autologous transplantation with blood-derived stem cells collected soon after the chemotherapy could be an alternative approach to allogeneic or autologous marrow transplantation

(p. 314-315).

The authors report a successful hematopoietic reconstitution using autologous cryopreserved peripheral blood cells from a patient with acute leukemia. The authors note that:

> Since the number of circulating HSC [hematopoietic stem cells] is low in normal humans [citation] and in patients with acute leukemia in remission [citation], the feasibility of autologous transplantation with blood-derived stem cells will depend upon the development of procedures to increase the number of progenitor cells that can be obtained by leukapheresis [citations].

(p. 314, col. 1).

and then continue to describe efforts to increase the number of "progenitor cells" collected.

To and Juttner, 1987, Brit. J. Haematol. 66:285-288 is a review that considers whether adult peripheral blood is a viable option for hematopoietic reconstitution of patients with acute myeloid leukemia (AML),[22] and thus represents this option as it was viewed by the art shortly before the filing date of the earliest priority application (Serial No. 119,746 filed November 12, 1987) for the instant application. To and Juttner discuss and describe the several questions that "need to be answered before PBSC [peripheral blood stem cell] autografting during first remission of AML can be recommended as a new therapeutic option" (p. 285, col. 1). These questions include the rate and completeness of reconstitution relative to the use of bone marrow, and whether there is malignant cell contamination among the collected PBSC (p. 285, cols. 1-2). The authors conclude that PBSC are efficacious in hematopoietic reconstitution of AML patients "if a sufficient CFU-GM dose is given" (p. 287, col. 1), which must be defined individually by each institution due to interlaboratory assay variations (see p. 285, col. 1); that the procedure is "safe and acceptable during first remission" and that "[t]he level of leukaemic contamination in PBSC collected during very early remission is probably low or absent," and suggest a phase II trial of autologous PBSC in first remission "to determine whether long-lasting remission or cure may result in a significant number of patients" (p. 287). Thus, the disclosed technique appears limited to collecting blood from AML patients in very early remission, the availability of the cells for transplant thus

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22 The authors disclose that peripheral blood stem cells for this purpose are collected "during periods of haemopoietic regeneration in very early remission from acute leukemia or after chemotherapy in lymphoma and solid tumours [citations]" (p. 285, col. 1).

being limited chronologically and by the patient's disorder and state of health. Additionally, having sufficient numbers of reconstituting stem cells appears to remain a problem, the spectre of malignant cell contamination still looms, and efficacy still remains to be conclusively established. Shortly before the filing date of the earliest application to which the instant application claims priority, the long-recognized needs in the art had not yet been fulfilled. This is also evidenced by the publication by Bell et al., discussed in the paragraph below.

Bell et al., (Oct.) 1987, Br. J. Haematol. 67:252-253, discloses that, with respect to the use of autologous adult peripheral blood mononuclear cells (PBMC) obtained from certain leukemia patients for use in attempted hematopoietic reconstitution, variations in the extent of the chemotherapy-induced progenitor cell (CFU-GM) overshoot means that some patients will yield collected PBMC with less than the minimum number of progenitor cells associated with successful hematopoietic reconstitution. To avoid the resulting problems of incomplete or unstable engraftment, or the need for intensive leucapheresis which is stated to be time-consuming and expensive, the authors propose the use of such PBMC in combination with autologous bone marrow transplants.

Subsequent to the disclosure of the invention of the instant application, numerous publications discussed the long-felt needs associated with prior art sources of stem cells for use in hematopoietic reconstitution and how the claimed invention of the instant application fulfills these needs. Such publications are discussed below.

Lynch and Brent, 1989, Nature 340:676, discusses the ways that cryopreserved neonatal blood could overcome some of the difficulties associated with matching donor and recipient using volunteer panels of bone marrow donors.

Chang et al., 1989, Bone Marrow Transplantation 4:5-9, documents that, even up through at least 1989, solutions to the problems of the GVHD associated with

allogeneic bone marrow transplantation[23] and the presence of cancer cells in autologous bone marrow transplantation[24] were still being sought.

Auerbach et al., 1990, Transfusion 30:682-687, note that "The advantages of using umbilical cord blood for transplantation, instead of using a small infant as a bone marrow donor, include avoidance of the risks of anesthesia and other potential complications associated with bone marrow donation" (p. 686, col. 1). Other publications disclose that: "collection of cord blood as described entails no intrinsic adverse effect or discomfort for mother or infant."[25] As stated by Broxmeyer et al., "[i]t was felt by all involved, and the human subjects institutional review boards of the involved centers, that the availability of cord blood in this case obviated the need for bone marrow aspiration from the infant sibling, although the infant sibling was available for bone marrow donation if necessary."[26]

McGlave, in a 1991 commentary,[27] also points out how the instantly claimed invention fulfills long-felt needs:

> Cord blood may offer advantages over bone marrow as a source of hematopoietic stem cells for related donor transplantation. Use of cord blood avoids a marrow harvest

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23 See p. 5, col. 1: "The necessity for HLA-matched bone marrow donors restricts the use of this approach to at best 15% of those with AML [acute myeloid leukaemia]."

24 See p. 5, cols. 1-2: "One major objection to autologous bone marrow transplantation (ABMT) without marrow purging is the potential presence of leukaemic progenitor cells in the marrow used for the transplant, which may be responsible for the recurring leukaemia. For this reason various methods have been used to purge autologous marrow before re-infusion. . . . The use of *in vitro* immunological methods for purging may be restricted by the known phenotypic heterogeneity of the leukaemic stem cells. . . . These *in vitro* data encouraged us to investigate the potential of LTBMC [long-term bone marrow culture] as a method of *in vitro* purging for ABMT."

25 Broxmeyer et al., 1990, Int. J. Cell Cloning 8(Suppl. 1):76-91 at p. 83.

26 Broxmeyer et al., 1991, Blood Cells 17:313-329 at p. 319.

27 1991, Blood Cells 17:330-337.

procedure which, although rarely associated with serious medical problems [citation], is costly, engenders some apprehension, and entails a hospitalization, general anesthesia, and often mild postoperative discomfort.

(p. 331).

He further states:

Unfortunately, due to the heterogeneity of HLA types in the United States, donors can be found for only approximately 20% of otherwise suitable unrelated donor bone marrow transplantation candidates [citation]. The problem of finding a suitably matched donor may be even more severe for candidates belonging to particular ethnic or racial groups, which are underrepresented in the NMDP [National Marrow Donor Program] [citations]. Even when an unrelated donor is available, the process of contacting and counseling the potential donor, obtaining and shipping blood samples for further typing, coordinating the hospitalization and harvesting process, and transporting large aliquots of viable marrow to transplant centers is time-consuming and costly. At present, the interval from initiation of an unrelated donor search to actual bone marrow transplantation averages 6 months [D. Stroncek, personal communication]. The process of locating and providing unrelated donor bone marrow can cost over $15,000.

If cord blood could be harvested and stored routinely, many of the problems associated with the current approach to unrelated donor transplantation might be overcome. The availability of stored cord blood would prevent the need to remove an adult related donor from the work force for a costly hospitalization and bone marrow harvest. An efficient and comprehensive collection system would allow salvage and storage of cells from underrepresented ethnic and racial groups, thus providing a source of stem cells for otherwise suitable candidates currently excluded from the unrelated donor bone marrow transplant process. Removal of time-consuming steps to locate and harvest adult donors could be lifesaving in the case of patients with rapidly progressing lethal hematopoietic disorders. In the case of a localized nuclear accident, such as the Chernobyl disaster, a repository of cord blood stem cells available for immediate use could provide the means for rapid transplantation of large numbers of lethally irradiated victims.

(p. 332).

Vilmer et al., 1992, Transplantation 53(5):1155-1157 state that:

> Allogeneic bone marrow transplantation has been advocated as a treatment for children with acute lymphoblastic leukemia who failed to achieve durable remission during chemotherapy. But the availability of an HLA-identical sibling donor or of a closely matched unrelated donor limits this option as do the poor results of HLA mismatched BMT [citation]. Human umbilical cord blood as a source of transplantable stem cells was proposed for HLA matched allogeneic reconstitution . . .

(p. 1155, col. 1).

Vilmer et al. subsequently state that: "[f]or the first time, this case report shows that HLA partially matched umbilical cord cells may be transplantable in a patient with advanced leukemia." (p. 1156).

Wagner et al., 1992, Blood 79:1874-1881 (of which co-inventor Broxmeyer is a co-author), states near the beginning of this article that "[u]nfortunately, suitable marrow is frequently not available. Either the patient's own marrow is contaminated with tumor cells, or potential allogeneic marrow donors are unsuitable on the basis of HLA disparity" (p. 1874). The authors close by saying that:

> human umbilical cord blood should therefore be viewed as an alternative source of hematopoietic stem cells. With thousands of children born each year, the routine collection and storage of human umbilical cord blood should at least be considered. The National Marrow Donor Program (NMDP) currently enlists approximately 480,000 volunteer marrow donors, yet suitable donors can be found in only 20% of otherwise suitable transplant candidates.[40] Typed banked cord blood would not only supplement the pool of registered marrow donors, especially for underrepresented ethnic and racial groups, but could also potentially shorten the time between the initiation of an unrelated donor search and day of transplantation. Until the creation of such a cord blood bank, the collection of cord blood should always be considered when a pregnant woman has a child with leukemia, lymphoma, marrow failure syndrome, or inborn error of metabolism who might potentially benefit from myeloblative therapy and the infusion of normal hematopoietic stem cells.

(p. 1879-1880).

Stone (ed.), 1992, Science 257:615, discloses that even as late as 1992, GVHD was killing more than one-fourth of marrow transplant patients, and that the problem of GVHD "may be alleviated by using umbilical cord blood, instead of bone marrow itself, as the source of the stem cells needed to repopulate the patients' marrow," resulting in efforts in the U.S. and abroad to set up the first cord blood banks.

Hows et al., 1992, The Lancet 340:73-76, further documents the long-felt needs fulfilled by the claimed invention:

> Cryopreserved human umbilical-cord (HUC) blood is an alternative to bone marrow as a source of haemopoietic "stem" cells for HLA-identical transplantation of children with leukaemia or Fanconi's anaemia . . .
>
> . . .
>
> Our findings indicate that the quality and quantity of HUC-blood-derived haemopoietic "stem" cells are better than those of normal bone marrow. . . .
>
> Two-thirds of patients with leukaemia and other life-threatening bone-marrow diseases lack HLA-identical sibling marrow donors, and must rely on finding histocompatible donors through volunteers' registries. [citation] Thus, many patients die or deteriorate before suitable transplant donors can be found. [citations] In addition, the risk of life-threatening acute graft-versus-host disease after unrelated marrow transplantation is high compared with the risk after HLA-identical sibling transplantation. [citation]
>
> . . . A bank of HUC blood would have advantages over existing volunteer marrow-donor registries because time-consuming donor tracing would be avoided, histocompatibility testing would be speeded up by testing with newly developed HLA-matching techniques [citations] samples of donated HUC-blood DNA held in reference files, and because HUC blood contains more naive T cells than normal adult bone marrow. HUC blood has high helper-suppressor activity, [citation] and thus has less potential for inducing graft-versus-host disease . . .

(p. 73).

Schiason, 1992, Bone Marrow Transpl. 9(Suppl. 1):93-94, similarly documents that the claimed invention fulfills long-felt needs:

> The benefits of using cord blood cells are numerous. This technology uses a disposable material, the cord blood. Anesthesia is unnecessary for the donor. There is no need for a transfusion for the donor and consequently no risk of viral transfusion. The graft can be performed earlier than with the marrow of the new sibling.

(p. 93).

Alby, 1992, Bone Marrow Transpl. 9(Suppl. 1):95-96 also states that: "BMT [bone marrow transplantation] with cord blood is a main progress sparing precious months. There are no more risks of harvesting on a small infant."

Charbord et al., 1992, Bone Marrow Transplantation 9(Suppl. 1):109-110, note that "[b]anking cord blood cells after HLA typing would make it possible to graft patients that are HLA compatible but family unrelated" (p. 109, col. 1).

Thierry et al., 1992, Bone Marrow Transplantation 9(Suppl. 1):101-104, also attest the long-felt needs for alternatives to bone marrow as a source of cells effective for human hematopoietic reconstitution, as of its date of publication (1992), and that cord blood appears to be solving these needs by providing additional potential donors of cells which can effect hematopoietic reconstitution with minimal GVHD:

> Although a successful therapy, allogeneic bone marrow transplantation has been limited by the lack of bone marrow donor. Only a small part of the patients who could benefit from hematopoietic transplantation can find an HLA identical donor in their family or in the registries of volunteers. There is a real need for other therapies for the remaining patients. It has been shown that the transplantation of HLA identical cord blood cryopreserved at birth can lead to total hematopoietic recovery with minimal GVH disease in familial situation [citation]. These early results show that the stem cells from one cord blood sample are sufficient to allow quick engraftment after standard conditioning. Although described first for Fanconi anemia treatment, patients with other diseases have been successfully transplanted with cord

> blood samples suggesting that umbilical cord blood could be used for the treatment of aplasias as well as malignancies in related or unrelated situations.
>
> We studied the possibility to set up a human umbilical cord blood bank as an alternative source of stem cells to bone marrow for hematopoietic transplantation.

(p. 101, first col.).

On page 102, they note that "[n]o adverse effects of the collection were observed for the mother or the neonate." And on page 103: "Cord blood collection is an easy and safe procedure." The authors conclude on p. 104 by stating:

> A frozen cord blood bank would augment the hematopoietic stem cell pool available for transplantation for the treatment of various hematopoietic diseases, thus supplementing the normal adult bone marrow donor registries and the available pool of related donors [citations].

Wagner et al., 1993, Blood 82(10)(Suppl. 1):Abstr. 330, note the "[e]ase of collection, absence of risk to the infant donor and low incidence of prior latent viral exposure" associated with the use of cord blood stem cells.

Ballantyne, May 6, 1993, The Times, discloses that bone marrow collection involves a two hour operation under anesthesia, which "leaves the donor bruised and unable to work for a week," in contrast to the collection of cord blood cells, which "is completely painless and non-invasive." The article also discloses the significant improvement in matching and locating donor and recipients for hematopoietic reconstitution associated with the envisioned use of frozen cord blood samples.

Vowels et al., 1993, N. Engl. J. Med. 329:1623-1625, also note the long-sought advantages afforded by the use of the claimed invention:

> Cord-blood transplantation has been performed in patients with Fanconi's anemia and leukemia, with sustained engraftment. [citations] This case report affirms the potential for transplantation of cord-blood stem cells to achieve sustained hematopoietic and lymphopoietic

> engraftment in patients with X-linked lymphoproliferative disease, thereby extending the potential use of cord-blood transplantation. We used cord blood to avoid subjecting an infant to a bone marrow collection. Although bone marrow has been collected successfully from very young donors, [citation] there are inherent risks that may be increased with decreasing age and size of the donor. [citation] These risks relate primarily to the blood volume of the donor. [citation] With cord-blood transplantation there is potentially no risk to the infant or the mother, although the cord is clamped earlier than usual.

(p. 1625, col. 1).

Rubinstein et al., 1993, Blood 81:1679-1690, provides a detailed review of the long-felt needs in the art, and their fulfillment by the claimed invention. Rubinstein et al. begin by summarizing as follows:

> Placental blood, as an alternative source of hematopoietic stem cells for BM [bone marrow] reconstitution, has recently been shown to yield successful sibling-donor placental blood "grafts" in children. [citations] Since, furthermore, the numbers of stem/progenitor cells in placental blood [citations] are in the range associated with successfully transplanted adult BM [citations], placental blood has the potential to overcome some of the limitations of the current system of registries for unrelated marrow donor procurement. "Banks" of cryopreserved placental bloods would not depend, for example, on the recruitment and continued collaboration of large numbers of volunteer potential donors and on compensating for the unavoidable attrition caused by retired volunteers. Systematic studies of the feasibility of using banked placental blood for BM reconstitution of unrelated recipients on a large scale seem, therefore, timely and warranted. At least three such studies have been proposed, including our own [citation].

(p. 1679, col. 1).

Rubenstein et al. then elaborate on the many ways cryopreservation of human neonatal/fetal blood would overcome the deficiencies long associated with the use of cryopreserved bone marrow:

> The difficulties inherent in registering volunteers, locating, testing, retyping, counseling, and eventually

extracting BM from unrelated donors, contrast with the ease and speed with which typed, tested, and frozen placental blood could be made available when needed.[25] Empirical demonstration that placental blood "transplants" are able to support the reconstitution of BM in an adult would give banked placental blood critical advantages over registered volunteer BM donors:

(1) Placental blood is an abundantly available and currently discarded source of hematopoietic stem and progenitor cells that can be harvested without risk to mother or infant.

(2) Ethnic balance can be maintained automatically in heterogeneous populations.

(3) Important infectious agents, particularly cytomegalovirus (CMV), are much less common in the newborn than in adults.

(4) Placental bloods, frozen and stored in a repository, can be made available on demand, eliminating the delays and uncertainties that now complicate the collection of marrow from unrelated donors.

(5) Frozen placental blood units are easily shipped and thawed for use when needed, whereas freshly donated BM has a limited shelf-life necessitating rigorous coordination between harvesting surgeons, transportation facilities, and transplantation teams.

(6) In a large inventory, placental blood units with common HLA types might be added only until an adequate number of replicates is frozen; further replicates could allow the selective culling of units, for example, by infectious disease risk status. Thus, the proportion of these common units would be kept to an optimum while that of uncommon units would continue to increase.

(7) There would be an undistorted accumulation of the HLA types encountered because, unlike volunteer donors who eventually retire from the Registry, stored placental bloods suffer no attrition other than by clinical use or by culling and substitution.

(p. 1679, col. 2 to p. 1680, col. 1).

Rubinstein et al. further elaborate on many of the above in their publication.

Newton et al., 1993, Exp. Hematol. 21:671-674, further confirms the long sought advances afforded by cryopreserved cord blood:

> **Introduction**. Bone marrow transplantation with marrow cells from a histocompatible related donor makes it possible to cure patients with hemopoietic malignancies, aplastic anemia or severe immunodeficiencies. In cases when a fully HLA matched and related donor is not available, two strategies may be adopted: seek a related donor incompatible for one or two loci or seek a fully HLA-compatible unrelated donor. In both situations, the risk of complications related to the transplantation (acute graft-vs.-host disease, graft failure, infections) is significantly higher than when a histocompatible sibling is available. The search for an unrelated donor requires an established listing of voluntary donors of known HLA phenotype, a costly procedure. Because of the unsatisfactory transplantation results obtained using unrelated donor marrow cells and of the difficulty in compiling a register, alternative sources of hemopoietic cells with reconstitutive ability similar to marrow cells have been eagerly sought.
>
> Since 1989 [citation] cord blood collected at birth has been used as a source of hemopoietic cells for bone marrow transplantation. . . .
>
> Since cord blood is an easily available material, it has been suggested that it might be used for adults as well as children [citation] and for HLA-matched recipients, both related and unrelated [citation]. Preliminary data suggest that cord blood cells may be less allo-reactive than marrow cells [citation]. If these clinical results are confirmed, it would be possible to engraft cord blood from an unrelated donor mismatched for one or two HLA loci at lower risk than for similar marrow allografts.
>
> These widening indications for cord blood transplantations would justify the constitution of cord blood banks of characterized HLA phenotype.
>
> No major ethical issues should be raised by the establishment of such banks since cord blood is discarded after birth. Now wasted, cord blood could become a highly useful material.

(p. 671, cols. 1-2, emphasis added).

Falkenburg et al., 1993, Ann. Hematol. 67:231-236, notes the long-felt problems associated with allogeneic bone marrow transplantation of finding an HLA-matched donor and GVHD, and how human cord blood is deemed to fulfill the long-felt need for an alternative source of cells capable of carrying out human hematopoietic reconstitution:

> Allogeneic bone marrow transplantation is currently the only curative treatment available for various hematologic disorders, including aplastic anemia, and certain inborn errors of metabolism [citations]. Major histocompatibility complex (MHC) matching between donor and recipient is important in the outcome of allogeneic BMT. HLA disparity between donor and recipient increases the incidence and severity of graft-versus-host disease (GVHD) after allogeneic BMT [citation]. In adults, the overall outcome of allogeneic BMT with bone marrow from haplo-identical donors is poor. Therefore, the possible use of unrelated HLA-matched bone marrow donors has been explored. Although HLA-matched unrelated transplants can be performed successfully, several problems, including severe GVHD, an increased risk of graft rejection, and the occurrence of non-Hodgkin's lymphoma after transplantation, have limited the use of these donors [citations]. Furthermore, the search for an unrelated donor may take between 2 and 6 months. Therefore, the availability of a cryopreserved "bank" of bone marrow grafts or other bone marrow repopulating cell populations for allogeneic BMT may increase the probability of finding a suitable HLA-matched donor.
>
> Human umbilical cord blood (UCB) may be used as an alternative source of bone marrow repopulating cells in allogeneic BMT [citations]. It has been demonstrated by Broxmeyer et al., and subsequently by others, that UCB cells can successfully repopulate young patients with Fanconi's anemia or leukemia, leading to sustained engraftment of donor cells [citations]. However, thus far no adult patients have been transplanted with single-donor UCB. Thus, although UCB contains stem cells, leading to immunologic and hematologic recovery after allogeneic transplantation, it is unclear whether sufficient bone marrow repopulating cells are present in UCB to transplant adult patients.

(p. 231, col 2).

The authors then state that their "results suggest that UCB may contain sufficient HPC for hematopoietic stem cell transplantation in adults" (p. 231, Summary, col. 1) and note the possibility that cord blood banks may "replace the requirements of adult volunteers as donors for unrelated bone marrow transplantation" (p. 236, col. 1).

Socié et al., 1994, Blood 83:340-344, notes the long-felt drawback associated with bone marrow transplantation of finding a suitable HLA-identical sibling donor or, failing that, an HLA-matched unrelated donor. The efficiency of finding an HLA-matched unrelated donor is stated to be only 20% (p. 340, col. 1). The authors note that, since "[i]n 1989, Broxmeyer et al [citing 1989, Proc. Natl. Acad. Sci. USA 86:3828] made the suggestion that neonatal blood retained in the placenta might contain sufficient hematopoietic stem cells to serve as a transplant inoculum" (p. 340, col. 1), greater than 20 such transplants have been performed (p. 340, col. 2). The authors note that "placental blood has the potential to overcome some of the limitations of the current system of registries for unrelated marrow donor procurement" (p. 340, col. 2) and suggest systematic studies of the feasibility of using cord blood banks to provide blood for hematopoietic reconstitution of unrelated recipients (p. 340, col. 2).

Wagner, 1994, "Umbilical cord blood transplantation, overview of the clinical experience," Blood Cells 20(2-3):227-233, discloses that "allogeneic bone marrow transplantation is complicated by a high incidence of acute and chronic GVHD and viral infection [citations]" (pp. 230-231, first paragraph under Discussion). The author states:

> In an attempt to reduce the morbidity and mortality associated with allogeneic bone marrow transplantation, clinical investigators in Asia, Australia, Europe and North America evaluated umbilical cord and placental blood as an alternate source of hematopoietic stem and progenitor cells for transplantation. To date, umbilical cord blood has been used to reconstitute hematopoiesis in 34 patients with a variety of malignant and non-malignant diseases treated with myeloblative therapy. . . . Of 23 evaluable patients with

> HLA-identical or HLA-1 antigen mismatched donors, none had grade 2-4 acute graft-versus-host disease (GVHD). In summary, these data suggest that umbilical cord blood is an acceptable source of transplantable hematopoietic stem cells, at least in recipients less than 40 kg and that the risk of acute GVHD is low.

(Abstract).

The authors also note that umbilical cord blood is rarely contaminated by maternal cells or latent viruses, that its collection is safe and harms neither mother nor child, and that it is possibly sufficient for engrafting adult recipients (p. 231, third full paragraph).

Issaragrisil, 1994, "Cord blood transplantation in thalassemia," Blood Cells 20(2-3):259-262[28], documents the long-recognized drawbacks associated with the use of bone marrow, which drawbacks can be avoided through the use of cryopreserved cord blood. The author notes:

> Thalassemias and hemoglobinopathies are prevalent in Thailand. Bone marrow transplantation can cure thalassemia but less than 30 per cent of the patients can have an HLA-identical sibling. Cord blood is an alternative source of stem cells for transplantation. . . . We report a successful cord blood transplantation in a patient with β-thalassemia/hemoglobin E disease.

(p. 2, Abstract).

Cryopreserved cord blood was used.

Kurtzberg et al., 1994, "The use of umbilical cord blood in mismatched related and unrelated hematopoietic stem cell transplantation," Blood Cells 20(2-3):275-284, documents the long-recognized drawbacks associated with the use of bone marrow for hematopoietic reconstitution (in particular, lack of a suitable donor, expense and delay in finding a suitable donor), which are alleviated by the use of

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[28] A copy of the manuscript as it was in press, rather than as published, is enclosed herewith, since Attorneys for Applicants do not presently have the manuscript as published in their possession.

cryopreserved cord blood. The authors note the long-recognized drawbacks associated with the use of bone marrow as follows:

> Up to 75% of patients in need of a bone marrow transplant lack an HLA-identical family member who could serve as their donor. Efforts to identify donors for these patients include the use of bone marrow from a haploidentical family member which is T-depleted to attempt to prevent or ameliorate the severity of GVHD [citation]. This approach, while successful in some patients, is complicated by a higher rate of graft rejection and delayed immune reconstitution which results in an increased incidence of fatal opportunistic infection and post-transplant lymphoma [citations]. The National Marrow Donor Program (NMDP) was established as a volunteer registry to identify unrelated living, adult volunteers who could serve as matched-unrelated-donors (MUD) for bone marrow transplantation [citation]. This organization has recruited >1 million volunteers and has successfully identified donors for approximately 26% of patients using the resource; however, the time required to complete a search averages >4 months and is frequently too long for a patient to wait. Furthermore, the search process is expensive and lacks sufficient participation of ethnic and minority groups [citations].

(p. 276, second paragraph).

The authors note the use of (cryopreserved) cord blood as an alternative to (cryopreserved) bone marrow:

> Over the past 6 years, umbilical cord blood (UCB) has emerged as an alternative source of hematopoietic stem cells for bone marrow transplantation (BMT) with approximately 40 transplants performed between related siblings to date [citations].

(p. 275).

Indeed, in the three patients subjected to cord blood transplantation by Kurtzberg et al.:

> All transplants were performed in high-risk patients who lacked a traditional bone marrow donor. Two of the three patients were of black and/or Hispanic origins and lacked a donor in the NMDP. The third was in relapse and had no time to complete an NMDP search. In all three cases, the prompt availability of a UCB unit greatly facilitated our ability to perform a timely transplant procedure.

(p. 280).

The authors report that hematopoietic reconstitution was achieved in all three patients, even though one patient received mismatched related cord blood mononuclear cells that had been cryopreserved, and two patients received mismatched unrelated cord blood that had been cryopreserved. The authors note that "[l]arge-scale and region-specific banking of unrelated UCB could allow for improved representation of minority group antigens in the donor pool as well as provide for more timely and less costly availability of donor units [citations]" (p. 282, first paragraph).

Vowels et al., 1994, "Use of granulocyte-macrophage colony stimulating factor in two children treated with cord blood transplantation," Blood Cells 20(2-3):249-254, documents the long-felt needs fulfilled by the use of cryopreserved cord blood:

> Even though a donor had been identified in an unrelated marrow donor registry, CBT [cord blood transplantation] was considered in patient 1 because the results with BMT [bone marrow transplantation] using unrelated donors are inferior to those seen with the use of a matched sibling [citation]. In patient 2 there was no donor in the extended family, and a registry search was not pursued when the possibility of a matched sibling (*in utero*) arose. The use of cord blood rather than collection of bone marrow from the infant after birth has a number of advantages. There is no risk to the fetus or mother in collecting cord blood, whereas blood [sic, marrow] collection involves an anaesthetic, multiple skin and bone punctures, and removal of blood volume during the marrow collection. In a large number of marrow collections, mortality has not occurred, but some adverse events have been reported [citation]. Furthermore, after cord blood collection, the infant is still available for marrow collection if the cord blood stem cells fail to engraft.

(pp. 252-253, second paragraph under Discussion).

Thompson, May 12, 1995, "Umbilical Cords: Turning Garbage Into Clinical Gold," Science 268:805-806 ("Thompson") describes the advantages of cord

blood over bone marrow stem cells, fulfilling a long-felt need in the art and providing surprising results, *e.g.*, in the higher proliferative capacity, less likelihood to trigger rejection and GVHD, and ability to be stored indefinitely to provide autologous transplants or transplants to unrelated recipients.

Stephenson, June 21, 1995, "Terms of engraftment: umbilical cord blood transplants arouse enthusiasm," Journal of the American Medical Association 273(23):1813-1815 ("Stephenson"), describes the "distinct advantages both logistic and biological, over bone marrow transplants" and the relative disadvantages of the traditional source of stem cell transplants, bone marrow. As described in the article, these advantages of the use of frozen cord blood stem cells over the prior art, fulfilling long-felt need and providing surprising results, include the following: ability of cord blood stem cells to be obtained noninvasively, with no risk to mother or infant, in contrast to the hospitalization and general or spinal anesthesia required for bone marrow extraction; cord blood is less likely to harbor infectious agents; cord blood stem cells can be stored for lengthy periods until needed by the donor or unrelated recipient; less stringency is required in matching cord blood stem cell donor and recipient; cord blood stem cells are more likely to provide engraftment and less likely to cause potentially fatal GVHD; and the availability of cord blood stem cells to treat the mother, *e.g.*, if she has a malignancy. Additionally, as Stephenson states, "[c]ord blood banks could provide a supplementary source for unrelated stem cell transplants" for those who cannot find a compatible bone marrow donor "and die or deteriorate while waiting for a match" (p. 1813, cols. 2-3).

Wagner et al., July 23, 1995, "Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease," The Lancet 346:214-219 ("Wagner et al."), describes the results of the first 44 consecutive patients treated by infusion of stem cells of umbilical cord blood and placental blood that had been

cryopreserved. Wagner et al. notes that "[a]llogeneic bone marrow transplantation [the prior art alternative to the use of the claimed invention] is an accepted therapy . . . [that] is limited by the availability of suitable HLA-compatible donors, risk of donor-host immunologic reactions, especially graft rejection and GVHD, and risk of opportunistic infection" (p. 214, col. 2). The article discloses that since September 1994, frozen stem cells of cord blood have been used to reconstitute hematopoiesis in 44 children with malignant and nonmalignant disorders. On the basis of the results of these 44 transplants, the authors conclude that "umbilical cord blood is a sufficient source of transplantable haemopoietic stem cells for children with HLA-identical or HLA-1 antigen disparate sibling donors with very low risk of acute or extensive chronic GVHD." The lack of maternal cell contamination in cord blood and numerous potential benefits of frozen, banked cord blood relative to bone marrow transplantation (immediate availability, no donor risk or donor attrition, very low risk of transmissible infectious diseases, lower risk of acute GVHD, greater ability to expand the available donor pool in minorities that are presently underrepresented in all marrow donor registries) (p. 218, col. 2) are also discussed.

Thomas, September/October 1995, "Hematopoietic stem cell transplantation," Scientific American SCIENCE & MEDICINE, pp. 38-47, is a current review of hematopoietic stem cell transplantation by Dr. E. Donnall Thomas, who won the Nobel Prize for his work in bone marrow transplantation (see also the boxed summary regarding Dr. Thomas on p. 41). Dr. Thomas, a recognized expert in the art, states that over 50 cord blood transplants have been reported, the results of which transplants have been "surprisingly good" (p. 44, col. 2, bottom paragraph), noting that "[a]lmost all of the transplants were successful, and the incidence of graft rejection was only 10%" (thus also acknowledging the surprising results provided by the claimed invention). The

cryopreservation of compositions comprising cord blood stem cells that is being carried out, particularly by the New York Blood Center, is noted (p. 45, col. 1). Dr. Thomas also notes the advantages in the use of cord blood cells over bone marrow cells: "Cord blood is an attractive source of hematopoietic cells for transplantation, not only because it is a byproduct of normal pregnancies but also because it can be collected from minority donors, currently underrepresented in the National Marrow Donor Program" (p. 45, col. 1). The foregoing provides additional evidence of the fulfillment of long-felt needs by the claimed invention. Dr. Thomas also acknowledges that it was the observations of Drs. Broxmeyer and Boyse (two of the inventors of the instant application) that led in 1989 to the first transplant using cord blood cells (p. 44, col. 2, bottom paragraph). The lack of utility of normal adult peripheral blood for hematopoietic reconstitution[29], in accordance with Applicants' remarks hereinabove, is also noted ("Many years ago it was demonstrated in mice and dogs that stem cells are present in the circulating blood, but in numbers too small to be of clinical value." p. 44, col. 2).

The evidence clearly and convincingly demonstrates that the claimed methods fulfill the long felt needs for methods of for hematopoietic reconstitution in patients with various diseases and disorders, using an easily accessible, abundant, inexpensive source of stem and progenitor cells that can effectively be used, without problematic GVHD. Consideration of the secondary criteria for nonobviousness clearly evidences the nonobviousness of the instant invention.

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[29] The situation with normal adult peripheral blood is contrasted with that occurring in peripheral blood during recovery from chemotherapy (or in peripheral blood from patients subjected to recombinant cytokines, recently appreciated in the art), also as explained in Applicants' Brief on Appeal (see particularly pp. 106-112).